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14. ABSTRACT

Repetitive mild TBI leads to chronic traumatic encephalopathy (CTE), but the underlying molecular changes remain unclear. Here, biochemical and genetic studies that deepen our understanding of the pathogenesis of CTE will be performed, facilitating diagnosis and therapeutic development.

15. SUBJECT TERMS

Tau, genetics, susceptibility, MAPT, chronic traumatic encephalopathy, Alzheimer disease

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INTRODUCTION:

Repetitive mild traumatic brain injury leads to neurological symptoms and chronic traumatic encephalopathy (CTE). The molecular changes underlying CTE are unknown, but our data demonstrate a spectrum of pathology with accumulation of aggregates of the microtubule-associated protein tau. Our preliminary data indicates an association between CTE and the tau gene (*MAPT*) H1 haplotype. How *MAPT* haplotypes contribute to CTE is unclear, but differences in transcription, mRNA splicing and translation may participate. The objective of this proposal is to validate the association of CTE with MAPT and characterize differences the expression of tau protein and tau-associated proteins in CTE patients. We hypothesize that the tau H1 haplotype increases CTE risk by increasing expression of abnormal tau. We will test this hypothesis by performing biochemical and genetic studies of CTE. Understanding the mechanisms that underlie changes in tau in CTE will enable biomarkers and treatments. These studies will deepen our understanding of how genetic and biochemical alterations in tau contribute to CTE, facilitating diagnosis and therapeutic development.

KEYWORDS:

Chronic traumatic encephalopathy, tauopathy, tau haplotype, MAPT, tau isoform

ACCOMPLISHMENTS:

What were the major goals of the project?

Our *long-term goal* is to identify molecular mechanisms regulating tau that can be used as diagnostics and to develop therapeutics for CTE. The *immediate goal* of this proposal is to correlate neuropathological findings with *MAPT* variation and discover differences in tau and tau-associated proteins in post-mortem brain, CSF and serum/plasma from CTE that will serve as potential biomarkers and facilitate future drug trials. We *hypothesize* that the neuropathological findings we are currently characterizing in individuals with CTE reflect molecular and genetic differences that will enable the development of biomarkers and therapeutics. To achieve these goals, we pursued the following specific aims:

Aim 1. To discover molecular signatures of CTE stage and severity.

Aim 2. To discover and validate candidate risk alleles for CTE.

Table 1. Major tasks from the statement of work

Specific Aim 1(specified in proposal)	Timeline	Site 1	Site 2	Status
Major Task 1: Histopathological correlates of CTE severity and progression	Months			
Subtask 1: Review of autopsy material, case selection, tissue retrieval	1-3	Dr. Crary	Dr. McKee	**Complete**
Subtask 2: Tissue embedding, sectioning and screening by histopathology	4-6	Dr. Crary	Dr. McKee	**Complete**
Subtask 3: Evaluation of neuronal, glial and inflammatory cellular changes	7-12	Dr. Crary	Dr. McKee	Ongoing
Milestone(s) Achieved: Completion of histopathological analysis	12			
Major Task 2. Tau isoform analysis				
Subtask 1. Review of autopsy material, case selection, tissue retrieval	25-28	Dr. Crary	Dr. McKee	**Complete**
Subtask 2. Tissue embedding, sectioning and screening by histopathology	29-31	Dr. Crary	Dr. McKee	Ongoing
Subtask 3. Tau immunoblots, ELISAs and EM	31-36	Dr. Crary		
Milestone(s) Achieved: Completion of biochemical analysis	36			
Specific Aim 2				
Major Task 3. To discover risk alleles for CTE				
Subtask 1. Review of autopsy material, case selection and DNA isolation	13-18	Dr. Crary	Dr. McKee	**Complete**
Subtask 2. MAPT haplotype analysis	19-21	Dr. Crary		Ongoing
Subtask 3. MAPT resequencing	22-24	Dr. Crary		
Milestone(s) Achieved: Completion of MAPT haplotype analysis	24			

What was accomplished under these goals?

Note: *This award was in the transfer process from Columbia University Medical Center to the Icahn School of Medicine at Mount Sinai. As such, no funds were available to conduct work until the transfer, effective on October 20th, 2016.*

The major activities related to this project were surrounding preparation of tissues for the histopathological studies and the initial genetic studies of *MAPT* haplotypes. Given the high interest in the genetics of CTE, the *MAPT* haplotype analysis, which had been planned for year three, was prioritized (see below).

1. **The work with post-mortem samples was approved by the Human Research Protection Office US Army Medical Research and Materiel Command on 2/16/2016 (Task 1, subtask 1).** This involved receiving approval from a representative from the Mount Sinai IRB and the determination was made that the project is IRB exempt. A letter that addresses the human subjects protections that are required was provided by the Chair of the Department of Pathology at Mount Sinai. Also, a description of the BU biorepository and collection of materials was prepared and submitted for review to the US Army Office or research protections.

2. Selection of biospecimens for the histopathological studies was completed at BU (Task 1, subtask 2) and preliminary histopathological characterization of the tau pathology was performed (Task 1, subtask 3). Brains from 20 individuals have been selected for immunohistochemical analysis. This includes 10 with CTE and 10 controls. Tissue has been embedded, sectioned and screened using immunohistochemistry

3. **Case material was reviewed, and tissue was selected for genotyping, and DNA isolated (Task 3, subtask 1) and initial ancestry analysis and *MAPT* haplotype analysis was performed (Task 3, subtask 2).** DNA was isolated from the brains of subjects with neuropathologically confirmed CTE. The *MAPT* haplotype was determined using haplotype tagging SNPs. Haplotype and allele frequencies were compared to population, autopsy and athlete controls.

4. FITBR refresher call was conducted with Miranda Osterheld to review data structures

Table 2. Patient data and assessments for histopathological studies

Study ID	Classification	Histopathological assessment
1	Pure CTE	Abundant macrophages, gliosis in white matter, several tangles
2	Pure CTE	Hemosiderin present in dilated vessels. No visible tau pathology or amyloid plaques
3	Pure CTE	White matter microgliosis, but healthy-looking vasculature and no tau pathology
4	Pure CTE	Abundant microgliosis and macrophages. No gliosis or dilated vessels
5	Pure CTE	Hypoxic ischemic encephalopathy, abundant macrophages
1	CTE + Aβ	Hypoxic ischemic encephalopathy
2	CTE + Aβ	Gliosis with macrophages in white matter. Plaque visible with several tangles. Vasculopathy.
3	CTE + Aβ	Hemorrhagic blood vessels with surrounding gliosis. Several tangles visible.
4	CTE + Aβ	Hemorrhages and old infarcts. Vacuolization of white matter with many macrophages. Visible tangles at sulcal depths.
5	CTE + Aβ	Slightly dilated blood vessels, but no gliosis. Some plaques but no apparent tangles.
1	Controls	Macrophages in white matter, but no tangles.
2	Controls	Severe vacuolization of the white matter with macrophages, but healthy blood vessels and no tangles.
3	Controls	Only mild gliosis, generally very healthy.
4	Controls	Lots of artifacts, mild gliosis, no NFTs
5	Controls	Very wide blood vessels, but no gliosis, macrophages, or tangles.
6	Controls	Heavy retraction around vessels and high amounts of cell shrinkage possibly due to hypoxia. Gliosis around vessels.
7	Controls	No diagnostic abnormality recognized
8	Controls	Some dilated vessels with gliosis. Possible pretangles around sulcus.
9	Controls	Hemosiderin common around vessels. No tangles or macrophages.
10	Controls	Vacuolization in layers 1-3. No tau pathology.

Histopathological studies of CTE

To prepare for these histopathological studies, we have collected and begun to characterize a collection of specimens (Table 2). Previously, we have observed a distinctive pattern of regional and cellular vulnerability with widespread accumulation of abnormal tau in the brains of military veterans and athletes with CTE (Goldstein et al. 2012; McKee et al. 2013). The changes in CTE are sufficiently distinct that we consider them diagnostic (Figure 1). Specifically, we see a spectrum of hyperphosphorylated tau pathology ranging in severity from focal perivascular epicenters of tangles, predominantly in the frontal cortex, to severe tau accumulation in numerous brain regions in CTE. As part of this proposal, we are further characterizing the

neuropathological findings in CTE and correlating them with disease severity, progression and military/clinical history. The pattern of cellular involvement in CTE is exceptional compared other neurodegenerative diseases. CTE patients display marked neuropil thread pathology. Both neurons and glia are involved, but whether these changes are occurring in neurites or glial processes, or both, is not clear (Figures 2 and 3). Glial tauopathy is not unusual, but the pattern in CTE is distinct with marked subpial accumulation of p-tau in glia in the sulcal depths and collections of astrocytes in deeper lamina, often surrounding blood vessels.

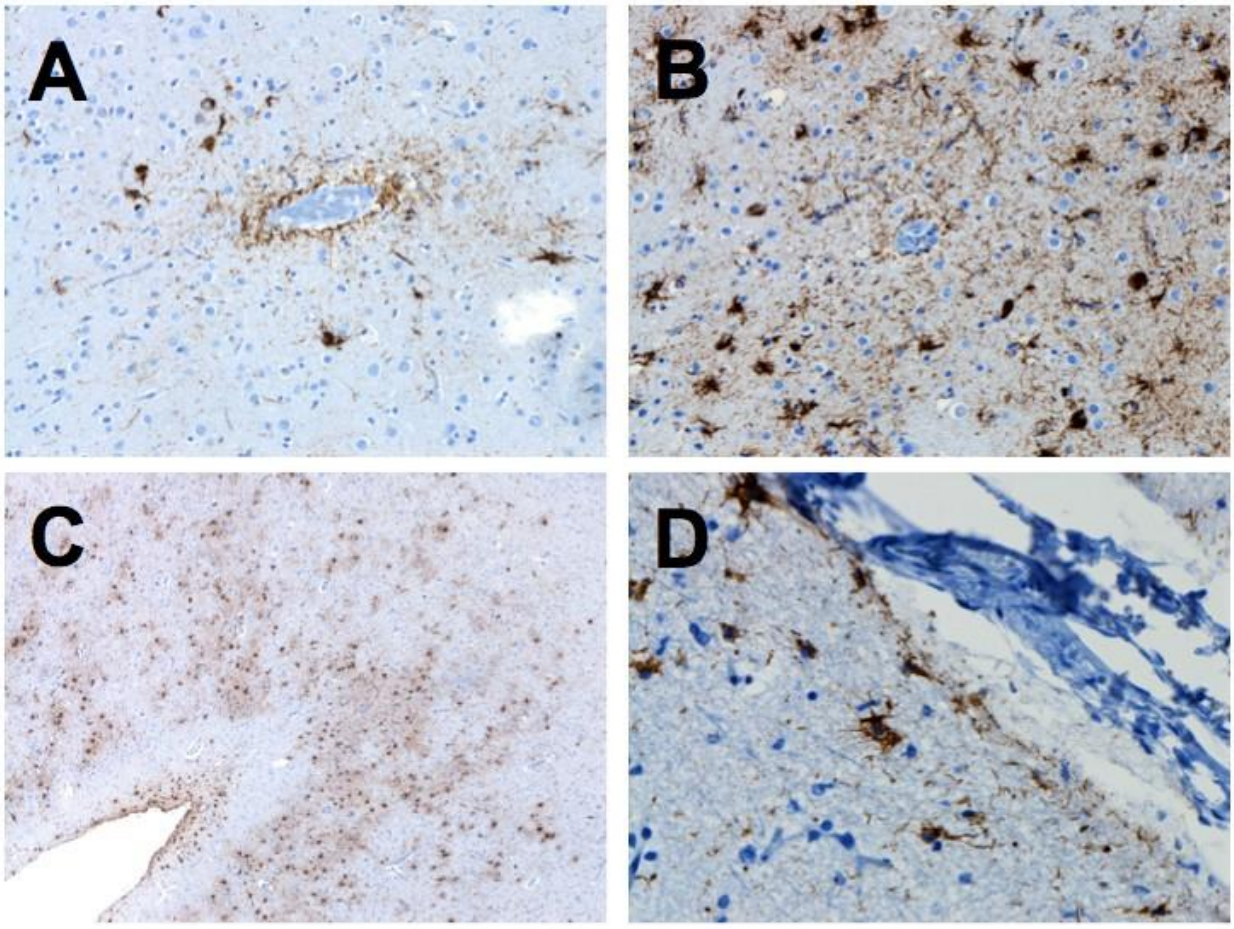

Figure 1. Tau pathology in chronic traumatic encephalopathy. Immunohistochemical staining using antisera targeting phospho-tau was used to screen tissue from subjects with CTE and reveals a spectrum of abnormalities. (A, B) The pathognomonic CTE lesion consisting of p-tau positive glia surrounding blood vessels in the cerebral cortex. (C) These lesions are commonly encountered in the depths of the neocortical sulci. (D) Subpial tau accumulation is also commonly encountered.

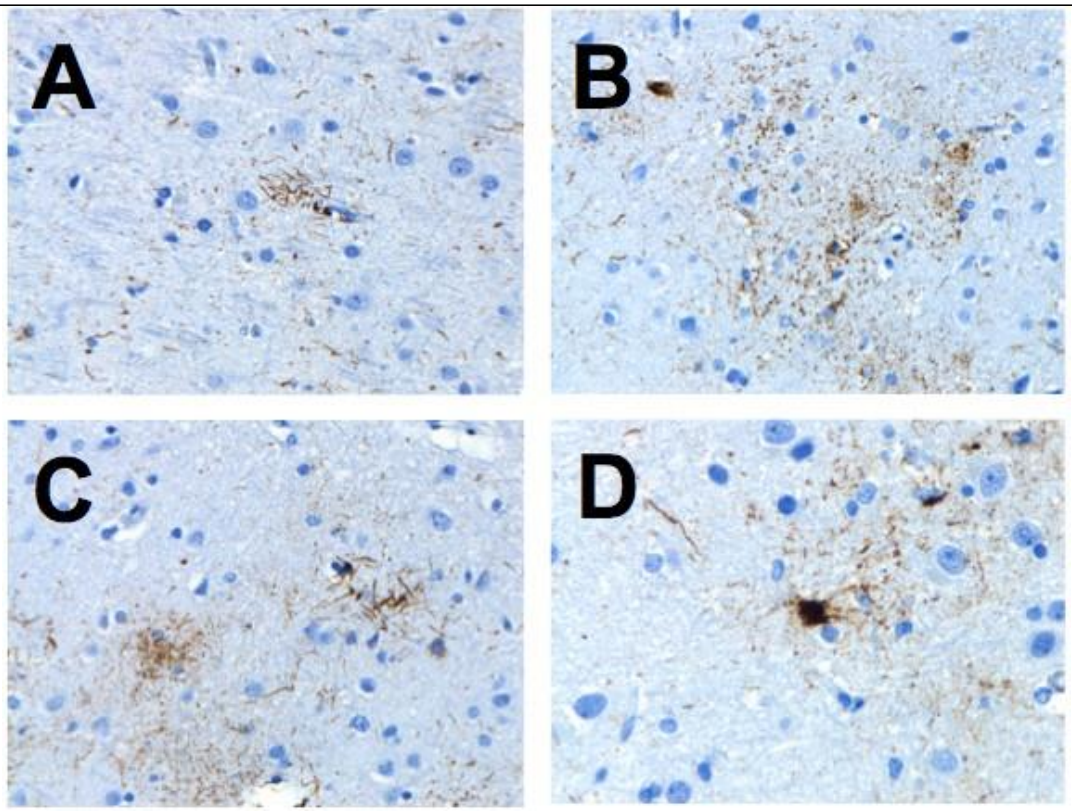

Figure 2. Glial tau pathology in chronic traumatic encephalopathy. Immunohistochemical staining using antisera targeting phospho-tau reveals a spectrum of glial abnormalities. (A) Fibrillary tau accumulation can be seen aroud blood vessels. (B, C, D) In addition, granular tau accumulation can also be seen in neocortical astrocytes.

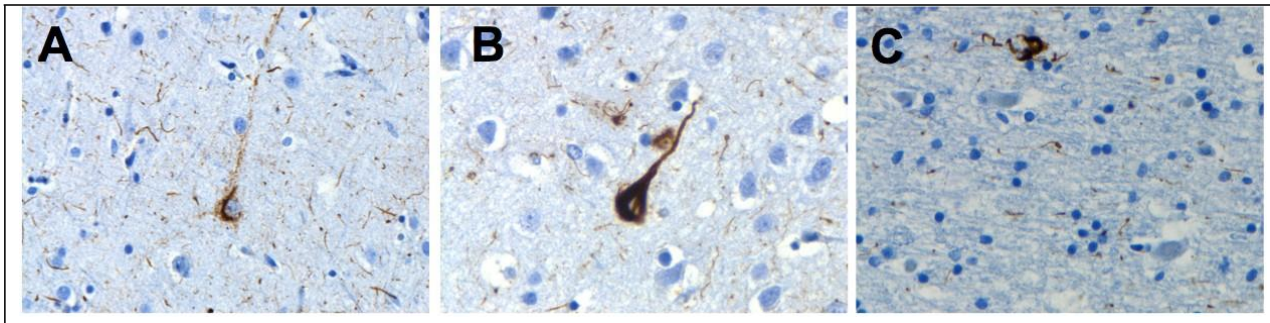

Figure 3. Neuronal tau pathology in chronic traumatic encephalopathy. Immunohistochemical staining using antisera targeting phospho-tau reveals a spectrum of additional abnormalities. (A) This example shows early granular tau oligomers present in a neocortical pyramidal neuron. (B) This neuron contains a mature intracellular tangle. (C) In the white matter, scattered small granular retraction balls can be seen.

Genetic studies of CTE

Our goal is to uncover common variation in *MAPT* and determine whether it is associated with CTE. In our first series of association analyses, we have begun to compare autopsy confirmed CTE patients with the control groups. Genotyping was performed on the Sequenom iPlex Massarray platform and statistical analyses in plink. The MAPT haplotype varies considerably among populations, and this can lead to hidden sample stratification, even among Europeans. To address this confounding variable, we performed an ancestry analysis using a minimal series of markers with modifications (Phillips et al., 2012) (Table 3). We uncovered good separation of European ancestry from African and Asian reference populations obtains from the 1000 genomes project reference panel (Figure 1).

Table 3. Ancestry marker panel design

Original SNP ID	Note	Proxy ID**	Distance	RSquared	DPrime	Arrays	Chromosome	Coordinate_HG18	Final design SNP ID
rs10141763									rs10141763
rs1024116									rs1024116
rs10843344									rs10843344
rs12913832									rs12913832
rs1321333	Design failure	rs6016294	1733	1	1	None	chr20	38281323	rs6016294
rs1335873									rs1335873
rs1426654									rs1426654
rs1498444									rs1498444
rs1573020									rs1573020
rs16891982									rs16891982
rs182549									rs182549
rs1886510									rs1886510
rs1978806									rs1978806
rs2026721									rs2026721
rs2040411									rs2040411
rs2065160									rs2065160
rs2065982									rs2065982
rs2303798									rs2303798
rs2304925									rs2304925
rs239031									rs239031
rs2572307									rs2572307
rs2814778									rs2814778
rs3785181									rs3785181
rs4540055									rs4540055
rs5030240									rs5030240
rs5997008	Design failure	rs356513657669	0.661	1		None	chr22	24687772	rs35651365
rs722098									rs722098
rs727811									rs727811
rs730570									rs730570
rs773658									rs773658
rs7897550	Design failure	rs350133802844	0.918	1		None	chr10	17102154	rs35013380
rs881929									rs881929
rs896788									rs896788
rs917118									rs917118

*** Philips et al., 2012, ** SNAP SNP Annotation and Proxy Search**

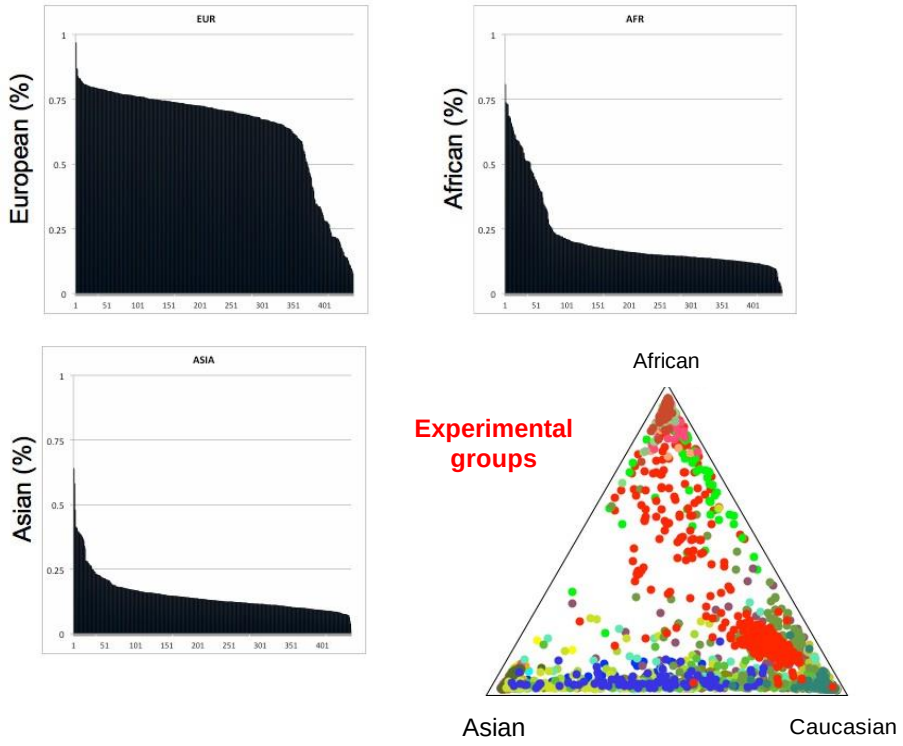

Figure 1. Ancestry analysis in CTE. The proportion of genomic material derived from European, African and Asian populations is estimated using the STRUCTURE ALGORITHM. These results were used to set thresholds control for sample stratification.

The ultimate goal is to validate our findings that CTE is associated with the *MAPT* H1 haplotype and to test known common genetic variation that exists on the H1 background that defines the H1 subhaplotypes, including H1c that has been implicated in other tauopathies. We determined the MAPT haplotype using a tagging SNP (rs9468) in autopsy confirmed CTE (*n*=146, average age 60, 17 – 98 years), autopsy controls without CTE (*n*=46, average age of death 41) and living control subjects without clinical evidence of neurological or cognitive impairment from the LEGEND study (*n*=79, average age 44, range 78 – 24 years). We also compared these allele frequencies to those in control populations from the NHLBI Exome Sequencing Project (*n*=6503) and the 1000 genome project (*n*=2504). An ancestry cutoff of 62.5% was selected and the genotype and allele frequencies are reported in Table 4. We found that the CTE cohort had an H1 allele frequency of 0.83, which was not significantly different from our autopsy, LEGEND or NHLBI controls. However, when compared to the European population norms from the 1000 genomes project, the H1 allele frequency was significantly increased in the CTE cohort. Subgroup analysis reveals that this difference was largely driven by the high frequency of the H2 haplotype in Italian and Spanish populations. Whether this represents a true association will be further tested in the next phase of the study.

Table 4. *MAPT* haplotype association analysis in chronic traumatic encephalopathy

		Control			1000 genomes (population controls)					
MAPT	**CTE**	**Autopsy**	**Legend**	**EVS**	**EUR**	**CEU**	**FIN**	**GBR**	**IBS**	**TSI**
Genotypes										
H1/H1 (*n*)	73	28	46	4608	290	59	80	48	58	45
H1/H2 (*n*)	26	8	26	1671	183	39	16	41	41	46
H2/H2 (*n*)	4	1	3	224	30	1	3	2	8	16
Total (*n*)	103	37	75	6503	503	99	99	91	107	107
H1/H1 (frequency)	0.71	0.76	0.61	0.71	0.58	0.6	0.81	0.53	0.54	0.42
H1/H2 (frequency)	0.25	0.22	0.35	0.26	0.36	0.39	0.16	0.45	0.38	0.43
H2/H2 (frequency)	0.04	0.03	0.04	0.03	0.06	0.01	0.03	0.02	0.08	0.15
Alleles										
H1 (*n*)	172	64	118	10887	763	157	176	137	157	136
H2 (*n*)	34	10	32	2119	243	41	22	45	57	78
Total	206	74	150	13006						
H1 (frequency)	0.83	0.86	0.79	0.84	0.76	0.79	0.89	0.75	0.73	0.64
H2 (frequency)	0.17	0.14	0.21	0.16	0.24	0.21	0.11	0.25	0.27	0.36
*Dominant model**		0.74	0.26	1.10	**0.02**	0.12	0.14	**0.01**	**0.02**	**0.00004**
*Recessive model**		1.20	1.34	1.00	0.57	0.39	1.04	0.80	0.41	**0.01**
*Alleles**		0.69	0.35	1.00	**0.02**	0.34	0.15	**0.06**	**0.02**	**0.000005**

* Two-tailed Fisher's exact test, CTE = chronic traumatic encephalopathy, EVS = Exome variant server, EUR = European superpopulation, CEU = Utah Residents (CEPH) with Northern and Western Ancestry, FIN = Finnish in Finland, GBR = British in England and Scotland, IBS = Iberian Population in Spain, TSI = Tuscans in Italy

What opportunities for training and professional development has the project provided?

Nothing to report (this is not a training or professional development grant)

How were the results disseminated to communities of interest?

We are scheduled to present preliminary genetic findings at the American Academy of Neurology meeting this April 22-28 (Boston). An additional abstract is being prepared for either the Alzheimer's Association International Conference or the American Association of Neuropathologists meeting.

What do you plan to do during the next reporting period to accomplish the goals?

The next phase of the genetic analyses will involve further analyses of our existing

genetic data and expansion of the cohort size. Our next round of genotyping will commence in early 2017 (total n=384). We will also perform additional analyses of the existing data, focusing on *MAPT* subhaplotypes and also considering *APOE* as an independent or interacting variable. The next phase of the histopathological studies will continue to address axonal injury markers, but also being to address neuroimmune and glial factors.

IMPACT:

*Nothing to report**

CHANGES/PROBLEMS:

Given the challenges with racial stratification of MAPT haplotype analysis, we are currently considering expanding our panel of ancestry markers to allow for stratification among European subpopulations.

PRODUCTS:

*Nothing to report**

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	*John Crary, MD-PhD*
Project Role:	*Principal investigator*
Researcher Identifier (e.g. ORCID ID):	JC2892 (era commons)
Nearest person month worked:	*1.2 (10% effort)*
Contribution to Project:	*Dr. Crary is overseeing the project.*
Funding Support:	*No additional support*

Name:	*Ann McKee, MD*
Project Role:	*Co-principal investigator*
Researcher Identifier (e.g. ORCID ID):	acmckee
Nearest person month worked:	*1.2 (10% effort)*
Contribution to Project:	*Dr. McKee is coordinating the collection of tissues,*

	neuropathological data and clinical phenotyping.
Funding Support:	*No additional support*

Name:	*Jesse Mez, MD*
Project Role:	*Collaborator*
Researcher Identifier (e.g. ORCID ID):	*NA*
Nearest person month worked:	*1.2 (10% effort)*
Contribution to Project:	*Dr. Mez has joined the*
Funding Support:	*Dr. Mez's effort is derived from a grant from the Alzheimer's Association to study the genetics of CTE.*

Name:	*Kurt Farrell*
Project Role:	*Post-doctoral*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*4 (29% effort)*
Contribution to Project:	*Dr. Farrell is coordinating the genotyping and histopathological studies at Mount Sinai Medical Center*
Funding Support:	*No additional support*

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

No.

What other organizations were involved as partners?

None

APPENDICES:

*Nothing to report**